IN THE CLAIMS

Please amend claims 1, 2, 4, 6, 7, 9, 11-13, 16, 17, and 19 as shown in the "Listing of Claims" below. Please cancel claims 8 and 10.

LISTING OF CLAIMS

Claim 1 (Currently Amended): A method for detecting pathogenic mycobacteria in clinical specimens, said method comprising the steps of:

(a) clarifying the clinical specimens from containment by conventional methods,

(b) treating the processed clinical specimens obtained in step (a) with a modified lysis buffer to inactivate live pathogenic mycobacteria to make the process safe for the user,

(c) extracting genomic DNA from the processed clinical specimen obtained from step (b) using a modified method to increase the yield and quality of DNA,

(d) designing sequence of SEQ ID ~~No.~~ NO: 4 from the DNA obtained in step (c) for specific detection of pathogenic mycobacteria, said designed sequence comprising of selected intergenic region of SEQ ID. ~~No.~~ NO: 3, a flanking region containing a portion of the gene mmaA1 of SEQ ID ~~No.~~ NO: 1 and a portion of gene mmaA2 of SEQ ID ~~No.~~ NO: 2 of the DNA obtained in step (c),

(e) designing and synthesizing a set of specific oligonucleotide primers of SEQ ID ~~No.~~ NO: 5, which is the forward primer and SEQ ID ~~No.~~ NO: 6, which is the reverse primer for Polymerase Chain Reaction (PCR) amplification of SEQ ID ~~No.~~ NO: 4,

(f) developing a PCR amplification process for specific amplification of SEQ ID ~~No.~~ NO: 4 of (d), said process comprising using the specific oligonucleotide primers designed and synthesized in step (e) for detecting presence of pathogenic mycobacteria in the clinical specimens, and

(g) ~~analysing~~ analyzing the amplified PCR product by restriction fragment length polymorphism (RFLP) analysis for differentiation of the species of the pathogenic mycobacterium for a quick assessment of HIV co-infection.

Claim 2 (Currently Amended): A method as claimed in claim 1, wherein the designed SEQ ID ~~No.~~ NO: 4 has a sequence as follows:

5'TGGATCCGTTGACCATGAGGTGTAATGCCTTTCCGGACCCTAGGTGGCCTTTCGGT
GCTTGCACGGAACGCACCGATGCTTCCCCCTCCCCGCATGCTCGAGGCATGCTATCC
GATACAGGGCCGCCGCACTAAACCGCGATCGAATTTGCCCAGGTCAGGGAACGGAT
ATGAGCGGACGAGCTACTTGGTCATGGTGAACTGGGCGACGTTGATTAGGCCTCTGC
GGAAGCGCTCCGCGCATCCGGTCAGATAGTGCATGAAGTTGTTGTAGACCTCTTCGG
ACTGTACGGCGATGGCGCGTTCGCGGGCAGCCTGTAGGTTGGCGGCCCATGCATCGA
GAGTCCGTGCGTAGTGGGAATTC 3'.

Claim 3 (Previously Presented): A method as claimed in claim 1, wherein the clinical specimen is selected from the group consisting of sputum, gastric lavage, cerebrospinal fluid, blood, tissue biopsies, bone marrow aspirates and other body fluids or tissues.

Claim 4 (Currently Amended): A method as claimed in claim 1, wherein clarification of the specimens in step (a) from the contaminants is carried out by adding to said specimens a digestion decontamination mix containing mild alkali, NaOH, tri sodium citrate and a mucolytic agent and guanidinium isothiocyanate in the range of about 0.4-2.5 M followed by concentrating the specimens ~~from~~ by centrifugation.

Claim 5 (Previously Presented): A method as claimed in claim 4, wherein the digestion decontamination mix containing mild alkali, NaOH, tri sodium citrate and a mucolytic agent and guanidinium isothiocyanate in the range of about 0.5-2.0 M.

Claim 6 (Previously Presented): A method as claimed in claim 1, wherein the DNA in step (c) is extracted from the treated clinical specimen using a modified lysis buffer by inclusion of ingredients comprising guanidinium isothiocyanate in a range of about 0.5-8 M, Tris.Cl pH 7.6 in a range of about 20-100 mM, N lauryl Sarcosyl in a range of about 0.5-2% by weight of the buffer, EDTA in a range of about 0.1-20 mM, β-Mercaptoethanol in a range of about 1-25 mM and NaCl is present in an amount of about 0.2M; and purifying the DNA to improve yield by thorough precipitation by organic solvents.

Claim 7 (Currently Amended): A method as claimed in claim 6, wherein guanidinium isothiocyanate is about 4M, Tris-HCl pH 7.6 is about 50 mM, N lauryl ~~Sacrocsyl~~ Sarcosyl is ~~about~~ 1% by weight of the buffer, EDTA ~~is about~~ 1 mM, β-Mercaptoethanol is about 10 mM and NaCl is about 0.2M.

Claim 8 (Canceled)

Claim 9 (Currently Amended): A method as claimed in claim 1, wherein the genomic DNA yield is increased ~~in the range of about~~ 25 to 50%.

Claim 10 (Canceled)

Claim 11 (Currently Amended): A method as claimed in claim 1, wherein high yielding amplification of DNA in step (f) is achieved by the modified Touch Down PCR cycling conditions, said conditions comprising steps of providing an initial high annealing temperature in the range of ~~about~~ 62-72°C followed by lowering of temperature in the range of ~~about~~ 0.1-1°C per PCR cycle for the first 10-25 cycles, then subsequently carrying out 30 PCR cycles at an optimum annealing temperature of ~~about~~ 56-62°C.

Claim 12 (Currently Amended): A method as claimed in claim 1, wherein high yielding amplification of DNA is achieved by modified Touch Down PCR cycling conditions, said conditions comprising steps of providing an initial high annealing temperature of ~~about~~ 70°C followed by lowering of temperature of ~~about~~ 0.8°C per PCR cycle for about first 14 cycles to about 58°C for another 25 PCR cycles.

Claim 13 (Currently Amended): A method as claimed in claim 1, wherein the oligonucleotide primers capable of amplification of intergenic region of SEQ ID ~~No.~~ NO: 4 for detection of pathogenic Mycobacteria in clinical specimens are selected from group consisting of:

a. 5' TGGATCCGTTGACCATGAGGTGTAATG 3' (SEQ ID ~~No.~~ NO: 5), which is the forward primer, and

b. 5' GGAATTCCACTACGCACGGACTCTC 3' (SEQ ID ~~No.~~ NO: 6), which is the reverse primer.

Claim 14 (Previously Presented): A method as claimed in claim 1, wherein the length of oligomeric primers is between 5 and 100 bases.

Claim 15 (Previously Presented): A method as claimed in claim 1, wherein the modified lysis buffer provides a cleaner preparation of the DNA.

Claim 16 (Currently Amended): A method as claimed in claim 1, wherein treatment with the modified lysis buffer containing 4M guanidinium isothiocyanate inactivates the live mycobacteria to make the procedure safer ~~fro~~ for the operator.

Claim 17 (Currently Amended): A diagnostic kit for the detection of pathogenic mycobacteria in clinical specimens, comprising primers selected from the group consisting of:

a. 5' TGGATCCGTTGACCATGAGGTGTAATG 3' (SEQ ID ~~No.~~ NO: 5), which is the forward primer, and

b. 5' GGAATTCCACTACGCACGGACTCTC 3' (SEQ ID ~~No.~~ NO: 6), which is the reverse primer.

Claim 18 (Previously Presented): A method as claimed in claim 1, wherein the contaminant clarified in step (a) comprises mucus and/or live organisms other than mycobacteria.

Claim 19 (Currently Amended): A set of primers of SEQ ID ~~No.~~ NOs: 5 and 6 comprising:

5' TGGATCCGTTGACCATGAGGTGTAATG 3' (SEQ ID ~~No.~~ NO: 5), which is forward primer;

5' GGAATTCCACTACGCACGGACTCTC 3' (SEQ ID ~~No.~~ NO: 6), which is the reverse primer.